THE EFFECT OF DRY COW THERAPY ON MASTITIS

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CHAPTER I

INTRODUCTION

Mastitis is responsible for one of the largest, if not the largest, economic losses in the milk producing industry. Annual losses in the United States due to this disease have been estimated to range from $225,000,000 to $1,000,000,000. These losses are the result of reduced milk production, abnormal milk composition, costs of drugs and therapy, increased replacement costs because of more severe culling, and increased labor to care for infected animals.

The fact that mastitis is present in virtually every milking herd exemplifies the severity of the problem. Most herds in which mastitis research has been conducted are found to have approximately 50% of the cows and at least 25% of the quarters infected. It is also noted that the level of infection is greater at parturition than at termination of the preceding lactation.

Staphylococcal and Streptococcal microorganisms cause at least 90% of all infections. These organisms gain entry to the mammary gland by way of the streak canal and teat sinus.

The mammary gland does have a circulating humoral immunity. These antibodies, called immunoglobulins, are present in relatively high levels in colostrum milk, but exist in very low concentrations in normal milk. Most efforts to establish an immunity to mastitis by the use of vaccines have failed or produced limited results.

Direct injection of antigens into the udder has resulted in severe udder irritation. Although immunity plays a role in preventing new infections, efforts to establish an immunity to most of the major mastitis causing pathogens is still in the research stage of development.

Sanitation, especially a post milking disinfectant teat dip, has been found to reduce the incidence of new infection. However, the overall infection status of herds is slow to decline when only sanitation is practiced. This is due to a large percentage of existing infections that persist from one lactation to the next.

An animal with an established infection may recover spontaneously or the infection may be eliminated by drug therapy, otherwise, the animal may be culled. The use of drug therapy during the lactating period to eliminate infections has been directed primarily towards quarters showing clinical symptoms of infection. This procedure does not always eliminate the infection. In addition, milk must be discarded for a certain period of time due to drug residues.

The treating of cows when lactation is terminated (i.e., when the cows are "turned dry") is presently advocated as an opportune time to eliminate infections and to prevent new ones from becoming established. Researchers in England and in the state of New York have shown remarkable reductions in the percent of infections at calving compared to the previous dry period when quarters were infused with an antibiotic in a long-acting base at drying off. They have also reported a significant reduction in new infections over the non-lactating period when dry cow therapy was used. However,

in studies involving several herds much variation in response to therapy was reported. This indicates that results of dry cow therapy may vary widely from one herd to another due to differences in the predominate pathogens, management and climatic conditions.

The objective of this study is to determine the effect of antibiotic treatment of quarters at drying off in eliminating mastitis and preventing new infections, given the dry climatic conditions and management systems of the Southwest.

CHAPTER II

REVIEW OF LITERATURE

Economic Losses

Janzen (1970) stated that the primary losses from mastitis were milk yield, milk composition, costs of drugs and therapy, and herd replacement costs. Dobbins (1972) found the value of discarded milk from 31 herds in a Georgia quality milk program was $2.37, $4.37, and $0.27 per cow, respectively, prior to initiation of a mastitis prevention program and six and 24 months afterwards. Total milk yield losses have been reported from 5% (O'Donovan, Dodd and Neave, 1960) to 25% (Landrey, 1966). Natzke et al. (1972) reported an average reduction in milk yield of 740 kg. per cow per year for each infection. Greatest losses of 878 kg. were observed in first lactation cows with infections by streptococci other than Streptococcus agalactiae. Coliform infections reduced milk by 654 kg., which was less than the reduction by any other infection.

Janzen (1970), in his review of milk composition losses, found cited losses in fat of 0.1 to 0.45%, not-fat solids 0.1 to 0.57%, lactose 0.1 to 0.77% and total solids up to 1.07%, due to mastitis.

Costs of drugs and therapy were reported by Marshall (1961) at $5.47 per cow, including milk losses, over a 40 week period. Fincher (1963) reported costs of $1.00 per cow for each farm visit in elim-

inating Streptococcus agalactiae infections. Hopkirk (1972) found the cost of antibiotics for 1,892 cows in 20 herds was $895.00 during lactation and $916.00 for dry period antibiotics for an average of $0.95 per cow per year. Dobbins (1972) reported costs for drug therapy at $0.96, $2.30, and $0.15 per cow, respectively, 12 months prior, six months after and 24 months after starting a mastitis prevention program.

Culling a cow is often the only successful means of eliminating an infection. Replacement costs due to mastitis average $22.00, $44.00 and $7.00 per cow prior to, 6, and 24 months after starting a prevention program (Dobbins 1972). Overall costs per cow per year for mastitis of $98.00 prior to starting and $36.00 24 months after initiating a control program were reported by Dobbins (1972).

Incidence of Infection

Criteria for Detection

Disagreement as to what constituted an infection is apparent in the literature prior to the publication of "Microbiological Procedures for the Diagnosis of Bovine Mastitis" by Brown et al. (1969). This publication, which was a project of the Research Committee of the National Mastitis Council, Incorporated, set forth guidelines for the collection of milk samples, culturing these samples, and identification of specific groups or species of mastitis organisms.

Major criteria listed in this publication include the use of the first stream of milk for culturing purposes, the use of a 70% alcohol teat end disinfectant, and the requirement that samples not

be held for greater than 24 hours prior to culturing. The authors set forth 35 to 37 C as the proper incubation temperature and a minimum of 50% relative humidity in the incubator. Bovine (preferably calf) or ovine blood is to be incorporated with agar as the growth media. A standard amount of milk, 0.025 ml, is streaked on culture plates which are held at incubation temperature for 18 to 24 hours before reading, then the plates are reincubated another 24 hours for further study. A minimum of 200 colony forming units (C.F.U.) per milliliter of milk, are required to designate a quarter as infected. Standard procedures are also stated for the identification of the more common mastitis causing microorganisms, and specific tests described to be used in the identification of each species.

Mechanisms of Infection

Periods of high exposure to potentially infectious organisms are between milking periods during the dry period, and before first parturition. These periods are associated with heavy contamination of the distal end of the teat canal (McDonald, 1970a).

A Summary of some of the reports since 1966 on cows and quarters infected at the beginning of the dry period is shown in table I. Similar infection rates have been found in lactating cows (Dodd et al., 1969; Kingwell et al., 1970; Philpot, 1969; Roberts et al., 1969).

It is widely accepted that the streak canal of the teat is the primary route of quarter infection. Fincher et al. (1956) found that after removing the soft keratin layer in the external portion of the streak canal, they could invariably induce infection by the

swabbing of Streptococcus agalactiae on teats of heifers. Neave et al. (1969) supported these findings, noting that orifice erosion increased infection rate. McDonald (1970b) found the keratin layers in streak canals of two susceptible cows were decreased in thickness, less dense, mesh-like and detached from the underlying epithelium in several locations. Morse, Hubben, and Mitchell (1970) demonstrated the bacteriostatic activity of teat canal lipid in vitro. They found an increased level of myristic acid with resistant keratins and an elevated content of stearic and oleic acids in those keratins in susceptible cows. Hibbitt (1970) further defined the role of bacteriostatic cationic proteins isolated from the keratin material in the streak canal. These proteins are able to inhibit growth of pathogenic strains of staphylococci and streptococci both in vitro and in vivo.

Dry Period Infections

Smith et al., (1966) reviewed the literature concerning udder infection during the dry period. Indications were that the level of infection was greater at parturition than at drying off. Spontaneous recovery eliminated very few infections and many new infections occurred during the dry period. A summary of some of the research on new infections during the dry period is presented in table 2.

Three aspects of preventing dry period infections have been described by Oliver, Dodd and Neave, (1956c) as follows: 1) destroy all pathogens remaining on the skin of teats after the last milking of lactation, 2) prevent any remaining bacteria from passing through the streak canal and, 3) prevent the growth of bacteria that gain entry.

TABLE I

REPORT OF INFECTION AT DRYING-OFF

Reference	Year	Cows		Quarters	
		Number	%Infected	Number	%Infected
Smith et al.	1966	350	50	...	...
Smith et al.	1967b	888	50	3552	25.4
Uvarov et al.	1967	...	...	384	23.4
Neave et al.	1969	621	62.5	...	...
Pearson and Wright	1969				
Trial II		...	...	366	25.6
Trial III		...	...	578	26.0
Trial IV		...	...	285	25.0
Natzke et al.	1972	...	...	...	28.1

TABLE II

NEW DRY PERIOD INFECTIONS IN UNTREATED QUARTERS

Reference	Year	No. of Quarters	%New Infection
Oliver et al.	1956d	300	15.0
Kingwell et al.	1967	...	11.1
Smith et al.	1967b	1144	9.5
Pearson and Wright	1969	565	14.0
Morse	1970	52	19.0
Bratlie	1972	91	5.9

Several researchers (Neave, Dodd, and Henriques, 1950; Oliver et al., 1956d; Thomas et al., 1972) have found that most new infections occur within the first 21 days of the dry period. Neave et al. (1969) stated that the quarter incidence of intramammary infection is at least ten times greater during the non-lactating period than during the lactating stage. Easier entry of pathogens through the streak canal because of lack of flushing by milking and lack of disturbance of pathogens in the streak canal and teat sinus have been postulated as causes of mastitis (Phillips, Whiteman, and Walker, 1969; Thomas et al., 1972).

Intramammary pressure, leakage of milk, concentration of bacterial inhibitors, composition of secretions and somatic cell counts did not appear to affect rate of new infection in work done by Thomas et al. (1972). These researchers also found that new infection rate was not affected by yield of milk at drying off, milking rates or teat patency. In contrast, the same group of researchers (Dodd and Neave, 1951) previously observed that new infections in the dry period occurred more frequently in fast milking cows. Oliver, Dodd and Neave (1956b) found an increase in rate of infection with increased milk yield at drying off. Dodd and Neave (1951) used rate of machine milking to measure teat patency and found that first calf heifers with peak milking rates of 6.79 pounds per minute had a new infection rate of 66.7% while heifers with a peak milking rate of 2.42 pounds per minute had a new infection rate of only 10.0%. Markos and Touchberry (1970) found significant ($P < .01$) positive phenotypic and genetic correlations, respectively, for maximum and initial rates of milk flow with milk yield. Thus, as selection is emphasized for increased milk production,

cows will tend to have more patent teats.

There was a significant positive relationship between leasions or chapped teats and new infection rate in herds practicing a full hygiene program in England (Neave et al., 1969). Method of drying off (abrupt versus intermittent) was found not to significantly influence rate of new infection (Oliver, Dodd and Neave, 1956a).

Immunity to Mastitis

Immunity to mastitis is a logical area of investigation considering the increase adversity to antibiotic residues in milk. Norcross and Stark (1970) listed three factors that complicate the establishment of an immune state to mastitis in the bovine: 1) Mastitis is caused by several groups of pathogens, several of which have a large number of species types. None of these appears to give cross immunity; therefore a large number of antigens are involved. 2) The mammary gland contents are an ideal environment for bacterial growth. Additionally, this media comes in direct contact with the environment twice a day. 3) The ability of the bovine to produce antibodies in response to antigenic stimuli by many antigens is not as good as other animals. The level of antibodies in the milk is not always the same or as high as that in the serum.

Three antigenetically separate classes of immune globulins have been described by Butler (1969). The globulin fraction, IgG, has been further characterized into fast IgG1 and slow IgG2. There is an apparent selective transport of IgG1 from the blood serum into the lacteal secretions (Dixon, Weigle and Vasques, 1961). This transport system increases the level of IgG1 in secretions prior to parturition independent of

serum IgG1 levels (Butler et al., 1972), and causes IgG1 to be the predominant immune globulin in lacteal secretions (Klaus, Bennett and Jones, 1969; Murphy et al., 1964; Porter, 1971).

Smith, Conrad and Porter (1971) found the milk whey protein, lactoferrin, present as a major compound in secretions of involuted mammary glands. This "red protein" has iron binding properties (Groves, 1960 and 1965). It is normally eluted with IgG1 on DEAE chromatography and does not precipitate with antibovine serum during immunoelectrophoresis as it is not present in serum (Masson and Heremans, 1966; Masson, Heremans and Schonne, 1969). Smith et al. (1971) postulated that lactoferrin may play a prominent role in the defense of the involuted mammary gland based on its bacteriostatic properties and its isolation in relatively large quantities (Masson and Heremans, 1966; Masson et al., 1969; Oram and Reiter, 1968).

Observations of Butler et al. (1972) that the amounts of IgG2 are equal to or greater than IgG1 in a bovine quarter with clinical mastitis suggest: 1) Inflammation resulting from the infection resulted in transudation of serum proteins so that IgG2/IgG1 ratio approached that of serum or 2) Local antigenic stimulation of lymphoid tissue in the area resulted in a local synthesis of IgG2.

Botes (1970) described the use of "Agrilabin" (a combination of one to 2.5 ml standardized immuno-globulin dissolved in 17.5 to 19.0 ml sterile normal saline solution plus 200,000 IU penicillin and 250 mg. dihydrostreptomycin per quarter) for the treatment of mastitis. Highly effective results were obtained against peracute, acute and chronic mastitis as well as subclinical cases. Dry cow therapy was particularly effective as a brief (two month) period of local passive immunity

appeared to be induced.

Sanitation

Plastridge (1958) in his review of mastitis noted that the main vehicles of mastitis transmission are 1) udder washing cloths and udder washing water, 2) milking machine teat cups, and 3) milkers' hands. More recently, researchers have recommended the use of separate single-service towels for the washing and drying of each cow and machine-stripping rather than hand-stripping. Sterilization of teat cups between cows has little additional benefit when a disinfectant teat dip is used after each milking.

Teat dipping (i.e., completely surrounding the teats with a disinfectant solution) is considered the single most effective procedure in the prevention of mastitis (Kingwell et al., 1970; Neave, Dodd, and Kingwell, 1966; Neave et al., 1969; Philpot, 1970; Roberts et al., 1969; Schultze and Smith, 1970). In spite of the conclusions of researchers that teat dipping is effective, considerable concern has been voiced as to which preparations have an acceptable efficacy. Oliver et al. (1956c) found that by dipping teats in a 5% iodine tincture solution for 20 seconds immediately after the last milking of lactation and 24 hours later, they could reduce new dry period infections by 23.8% in the cows and 8.0% of the quarters. Both of these treatment differences were significant ($P < .05$).

The effect of teat dipping on the reduction of mastitis in lactating cows was studied by Wesen and Schultz (1970). They found a

commercial iodine teat dip preparation (Bovadine[1] 10,000 ppm) reduced new streptococci and Staphylococcus aureus infections by 53.2% over non-dipped controls. They found no apparent reduction of infection due to coliform bacteria (gram-negative rods) by the use of teat dipping. Philpot (1971) conducted a comparative study of teat dips. He compared chlorine (4% chlorine, prepared by addition of one quart of water to one gallon of Clorox[2]) to three commercial iodine solutions (Mastimin[3], Bovadine[4], and Teat Guard[5]) in three separate trials. Control animals showed no reduction of new infection in the trials, whereas the 4% chlorine produced an average reduction of 82% and the iodine compounds a 75% reduction. Some teat irritation with 4% chlorine was noted during the initial phases of each trial. Marshall, Sikes and Morgan (1969) compared an acid-type iodophor teat dip at levels of eight percent and two percent phosphoric acid, and a product containing hexachlorophene that was sprayed on the teats; with no treatment as mastitis preventatives. There were no differences among the three treatments, but clinical mastitis cases were 52% fewer in disinfected quarters than in nontreated controls. Kingwell et al. (1970) found a 67% reduction of infections after two years of using a 4% available chlorine teat dip in addition to dry cow therapy. Neave et al. (1969), citing date

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[1]Lazarus Laboratories, Inc. Division of West Chemical Products, Inc. Long Island City, New York.

[2]Clorox Company, Oakland, California.

[3]Diversey Chemical Company, Chicago, Illinois.

[4]Lazarus Laboratories, Inc. Division of West Chemical Products, Inc. Long Island City, New York.

[5]Klenzade Products, Division of Economics Laboratory, Inc., St. Paul, Minnesota.

reported elsewhere (Report 1957. National Inst. Res. Dairying, Shinfield, p. 71.) stated that on the basis of a comparison within cows, post-milking teat dip [0.5% chlorhexidine (Hibitane) in 75% ethanol (w/v)] reduced new streptococcal infections sevenfold.

Schultze and Smith (1970) tested the effectiveness of chlorhexidine at 0.2% concentration to reduce the apical teat microflora. They found a 95% reduction of gram positive cocci at the apical teat end by use of this teat dip. Additionally, during a 31-week challenge by S. areus in teat cups, they found nine new infections in controls and three new infections in teat dipped quarters. However, the average onset of infection for the dipped teats was delayed 56 days longer than the average onset in the controls. In a later study, Schultze and Smith (1972) found a 95, 87 and 67% reduction in apical teat microflora, respectively, for chlorhexidine (0.2%), iodophor (1.0%) and hypochlorite (4% available chlorine). There was no reduction in efficacy with repeated use of any of these compounds.

Weckbach and Langlois (1972) found evidence that "Bovadine" teat dip may alter the normal characteristics used to identify staphylococci. In samples of foremilk from treated and untreated glands significant differences ($P < .05$) were found in the percent of isolates positive for coagulase, lysozyme and phosphatase tests. A possible explanation was that "Bovadine" acted as a mutagen causing mutant strains of staphylococci to develop.

Phillips et al. (1969) reported trials on half udders to determine the effect of "stripping" (removal of contents of teat sinus prior to udder preparation) in reducing the number of clinical and sub-clinical infections when teats were pre-inoculated with pathogenic bacteria.

By removal of teat sinus contents, after wetting the udder but prior to washing or drying, they postulated that the risk of spreading bacteria to more favorable incubation sites in the udder would be greatly reduced. Using 540 cows in four herds, in which two quarters of each cow were controls, they found a significant reduction ($P < .05$) in the number of new infections except in 67 cows of one herd. In this herd the treatments were reversed at two month intervals creating three treatment periods. The third period had no significant differences ($P > .18$) for the treatments. Later, Frost and Phillips (1970) found similar results in that a significant ($P < .01$) reduction in number of quarters developing mastitis, compared to control quarters, was found by using the above "stripping" technique. They also noted that failure to machine strip a quarter during the milking prior to purposeful infection, resulted in a significant increase ($P < .01$) in clinical mastitis.

Dry Cow Therapy

The nonlactating period has been advocated by several researchers as the most opportune time to reduce the number of existing infections and prevent new ones (Dodd et al.,1969; Natzke, 1971; Pearson and Wright, 1969; Philpot, 1970; Smith et al., 1967b). Several reasons have been cited for this increased efficacy: 1) dry cow therapy contaminates no saleable milk; 2) it prevents most new infections from occurring during the dry period; 3) it allows the regeneration of damaged udder tissue prior to calving; 4) high persistency antibiotics may be used; and 5) it provides that a much higher proportion of cows are likely to be free from infection at the time of highest milk yield.

Dry cow therapy was first reported by Moak in 1916. Pearson (1951) used a combination of penicillin in an oily suspension to prevent the Corynebacterium pyogenes variety of "summer mastitis." Pearson suggested using 100,000 units of calcium penicillin in an oily base as a series of multiple injections every 14 to 18 days during the susceptible summer months. He used within cow controls and found a 92% actual protection with this method.

Smith et al. (1966) reported that earlier workers were able to eliminate Streptococcus agalactiae by use of penicillin G in the dry period, but that S. aureus infections were difficult to eliminate. S. aureus is known to develop resistance to penicillin by the production of penicillinase, an enzyme that destroys penicillin. It is also known that a S. aureus infection will wall itself off in the udder making it difficult to reach with antibiotics. Because of these peculiarities, Smith et al. (1966, 1967b) conducted experiments with dry cows using cloxacillin, a semi-synthetic penicillin. In their preliminary studies (Smith et al., 1967a) cloxacillin as a benzathine salt was more persistent than the sodium salt. A field trial involving 35 herds and 888 cows was conducted to test the effectiveness of two forms of cloxacillin for dry cow therapy (Smith et al., 1967b). Treatments were 1g cloxacillin as the benzathine salt in each quarter, 0.2 g cloxacillin as the sodium salt, and a control. The groups receiving antibiotic therapy were also teat dipped in a hypochlorite solution (5% available chlorine), but the controls were not teat dipped. About 50% of the cows had infected udders at the start of the trial. One gram of cloxacillin at drying off reduced infections to 15.6% at calving, whereas 0.2g sodium cloxacillin reduced infections to 26.0%.

The controls increased to 62% at calving. There were 9.5% of the control quarters that developed new infections over the dry period, while 3.3% and 1.7% of the treated quarters developed new infections.

Pearson and Wright (1969) tested the effectiveness of several antibiotics as dry cow treatments in four trials. In trial I, 60% of the staphylococcal infections were cleared by infusion of 200 mg sodium cloxacillin at drying off, whereas an average of 35% of all nontreated infections recovered spontaneously. In trial II a 75% clearance of staphylococcal infections was obtained with 200 mg sodium cloxacillin. A 100% clearance of streptococcal infections was obtained with 300 mg procaine penicillin G. Combined staphylococcal and streptococcal infections were treated with 250 mg novobiocin in a mixture with 300 mg procaine penicillin G, with a clearance rate of 75%. In trial III use of the above combination of novobiocin and penicillin resulted in clearance rates of 64% and 100% for staphylococci and streptococci organisms respectively. No teat dip was used in any of the first three trials.

In trial IV, where untreated controls, consisted of leaving half of the udder untreated, 500 mg benzathine cloxacillin produced clearance rates of 56% and 100% for staphylococcal and streptococcal infections, respectively. All teats were dipped in an iodophor compound at drying off in this trial. Spontaneous clearance rates of 39% for staphylococcal and 44% for streptococcal infections were observed.

Kingwell et al. (1967) compared two levels of benzathine cloxacillin, 0.5g and 1.0g. Both were equally effective in eliminating infections. A 4% hypochlorite teat dip solution was used for all cows. New infections at calving were found at the rate of 3.6% in both

treatment groups. Kingwell et al. (1970) reported results on the first two years of a three year study in which they used 0.5g benzathine cloxacillin and a 4% hypochlorite teat dip in combination with a partial hygiene system and teat dipping only to reduce mastitis infections. Preliminary results were an equal reduction of 67% in infected quarters in both the partial hygiene and teat dipping only treatments.

Dodds et al. (1969), in studies with chronic or resistant mastitis herds, used 200 mg of sodium cloxacillin in a 3% aluminum monstearate base for treatment of lactation and dry period mastitis. One week after calving 82% of the quarters infected at drying-off were found to be bacteriologically negative.

Uvarov et al. (1967) reported a 78% average clearance of infections over the dry period resulting from the use of 300 mg penicillin G and 250 mg novobiocin (as a monosodium salt) in a 3% aluminum monostearate base. Rosenzuaig and Mayer (1970) found a 61.4% clearance of staphylococcal infections during the dry period as a result of infusing benzathine cloxacillin at the rate of 0.5g per quarter and a teat dip containing 5,000 ppm iodophor.

Bratlie (1972) used for treatments and a control group to study the effectiveness of dry cow therapy on mastitis as follows: 1) neutral ointment base; 2) cloxacillin; 3) "drying off ointment" from Apothekernes Laboratorium, Oslo; 4) Solumast Pfizer; and 5) nontreated controls. There was a spontaneous recovery rate of 60% in the group treated with the neutral ointment base. In treatments 2, 3, and 4, recovery rate from mastitis was 80%, of which only 20% can be attributed to treatment. There was no increase in prevention by treating uninfected quarters prior to drying-off.

Natzke et al. (1972), in a comprehensive field study of dry cow therapy and teat dipping in New York state, found a reduction of 75% of infections over the dry period. They used one million units of penicillin with 1.0g dihydrostreptomycin and a teat dip of 4% chlorine as a treatment in 24 commercial herds over a period of three years. No controls were used in this work.

A great deal of variation in response to dry cow therapy has been observed, especially in those studies that involved several commercial herds (Kingwell et al., 1970; Pearson et al., 1969; Roberts et al., 1969). Also, only limited research has been conducted on dry cow therapy in the drier climates of the southwestern United States and other dry areas of the world to determine the climatic and management effects of these areas on the efficacy of dry cow therapy.

CHAPTER III

MATERIALS AND METHODS

Experimental Plan

Animals from the Oklahoma State University dairy herd were used for the experiment. A randomized block design was employed, with blocking on status of infection (negative or S. aureus) and by lactation number (one, two, three or more) near the end of lactation (Table III). Within each block, animals were assigned to a treatment by pairs in a randomized sequence in which the first available animal was assigned to the control group. When several animals were available at the same time for assignment to a particular block, the cow with the lowest neck chain number was assigned to the first available treatment and further assignments progressed numerically upward. Cows with no S. aureus, but with other infections were omitted from this experiment.

Treatments

The two treatments were: 1) control, no infusion and 2) an intramammary infusion of 500 mg benzathine cloxacillin[1] (a semi-synthetic pencillin in a long-acting peanut oil vehicle) into each quarter of designated cows after the last milking of lactation.

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[1]Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, New York.

TABLE III

RANDOMIZED BLOCK DESIGN OF EXPERIMENT

Item	Lactation number	Treatment
Negative cows	One	C[a] T[b]
	Two	C T
	Three or more	C T
S. aureus infected cows	One	C T
	Two	C T
	Three or more	C T

[a]Nontreated controls.

[b]Treated with 500 mg benzathine cloxacillin per quarter at drying off.

Teat dipping of cows with an iodine solution[2] (10,000 ppm) once a day for one week after drying-off was initiated after the first 40 cows had been put on experiment. Consequently the remaining 43 cows from which data has been collected were teat dipped for one week after drying-off.

Management of Cows

Experimental animals were group fed in an open, dry lot and milked either in a double "three-in-line" parlor or in stanchions. Animals were milked in different systems due to the renovation of milking facilities shortly after initiation of the experiment.

Dry cows were fed and housed similar to, but separate from, the milking herd, or were allowed to graze native pasture. Approximately one month prior to expected calving date, dry cows were grouped near the milking facilities and accustomed to the milking herd ration. Periodic inspections were made of the dry cows throughout the dry period for indications of clinical mastitis, such as abnormal swelling, redness, or hardness of the udder.

A standard management program was followed during the entire experiment. This included the proper maintenance of milking equipment, dipping of teats with a recommended disinfectant[3] after each milking, the use of a strip cup to detect clinical mastitis during lactation, treatment of clinical cases during lactation and the use of acceptable milking technique.

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[2]"Bovadine," West Agro-Chemical Products Inc., Subsidiary of West Chemical Products Inc., Long Island, New York.

[3]Ibid.

Collection of Data

One week prior to drying off, samples of foremilk from each quarter were examined bacteriologically for presence of mastitis causing pathogens. Quarter samples were also taken 4 to 10 days after freshening, one month after freshening and whenever clinical mastitis was detected on the strip cup during lactation, or suspected in the dry period. Quarter samples at drying off, freshening, and one month after freshening were taken in a series of three, all within the time span of one week. The first quarter sample consisted of a single sample of foremilk from each quarter taken at either a Tuesday or Thursday morning milking. The second and third foremilk sample were collected the following Tuesday or Thursday morning as a duplicate sample in which the teat ends were thoroughly scrubbed between samples and both samples were taken prior to attachment of the milking machine. When clinical mastitis was detected a duplicate sample, as described above, was taken prior to attachment of the milking unit or treatment.

Infected quarters were defined as those showing 200 or more colony-forming units per ml of secretion, as defined by the Research Committee of the National Mastitis Council, Incorporated (Brown et al., 1969). A quarter was recorded as being infected only when the same pathogen was isolated from two consecutive samples.

Laboratory Procedures

Foremilk samples (including the first stream of milk) were collected aseptically from each quarter into sterile 18 x 150 mm glass test tubes with metal caps. Udders were washed with warm water and

dried with individual paper towels. The teat end was scrubbed with 70% ethanol prior to sample collection. Samples were cultured the morning of collection. After mechanical agitation of tube contents, 0.02 ml of milk was streaked onto 5% bovine blood agar in a 10 cm diameter petri plate. Petri plates were appropriately marked with the date, cow number, quarter number, type of sample (drying-off, freshening, one month post-partum, or clinical sample), and designated as to original single sample or the first or the second of the duplicate samples. These were incubated at 37C for 18-24 hours. After this period of incubation, an individual bacteriological report was initiated for each cow and for each of the three samples per cow, i.e., original, first and second duplicate samples. The number of colonies was recorded and the plates incubated for another 24 hours. Further observations were made after this additional period of incubation. Procedures followed to identify specific groups or species of mastitis-causing microorganisms were those stated by Brown et al. (1969), p. 10-27.

Specific laboratory tests performed to identify the common mastitis streptococci included the gram stain for gram-positive chains of spheres, the CAMP-esculin test in which a medium of blood agar with a 0.1% esculin and 0.1% ferric citrate was used, and the test for ability to hydrolyse sodium hippurate. In the CAMP-esculin test a culture of S. aureus capable of producing a large zone of alpha hemolysis was streaked across the center of the plate containing blood agar esculin-ferric-citrate medium. Pure culture isolates, selected from blood agar plates, were streaked perpendicular to and within 2 to 3 mm of the staphylococcus inoculation.

Inoculated plates were incubated at 37C for 18 to 24 hours and the cultures examined for CAMP reaction, esculin splitting, and hemolysis. A positive CAMP reaction occurred when there was a semicircular zone of complete lysis in the alpha zone of hemolysis produced by the staphylococci. A positive esculin splitting culture was one that shows browning of the medium around the streptococcal inoculation. The test for hydrolysis of sodium hippurate was routinely conducted. An acidified solution of 12% ferric chloride was prepared, and 0.5 ml was slowly added to a clear supernatant fluid of the culture in a Wassermann tube. This combination was mixed immediately and a final interpretation made after 10 to 15 minutes. A distinct presistent brownish percipitate was recorded as positive evidence of hydrolysis of sodium hippurate to benzoic acid. Additional characteristics of the more common mastitis causing streptococci are presented in table IV.

S. aureus is the most common staphylococcal microorganism causing mastitis. It normally can be identified by creamy, grayish-white colonies on blood agar with typical zones of hemolysis. Staphylococci are gram-positive spheres in pairs or clusters when observed microscopically using the gram stain.

The coagulase test will distinguish S. aureus from the nonpathogenic Staphylococcus epidermidis. For this test a colony from a 24-hour blood agar culture was emulsified in 1.0 ml of fresh citrated rabbit plasma diluted 1:5 with physiological saline. Positive and negative controls were performed at the same time. The diluted colonies were incubated in a water bath at 37C and examined for clotting of the plasma after 1, 2, 4, 8, and 24 hours of incubation. Any degree of clotting of the plasma was accepted as confirmation for S. aureus.

TABLE IV

DIFFERENTIAL CHARACTERISTICS OF STREPTOCOCCI MOST COMMONLY CAUSING BOVINE MASTITIS[a]

Organism	Lancefield group	Hemolysis	Hydrolysis of: Esculin	Hydrolysis of: Sodium Hippurate	Acid produced in broth containing: Lactose	Sucrose	Salicin	Mannitol	Raffinose	Inulin	Trehalose	Sorbitol
Primary importance												
S. agalactiae	B	β (narrow) α ν	-	+	+	+	±	-	-	-	+	-
S. dysgalactiae	C	α ν	-	-	+	+	-	-	-	-	+	±
S. uberis	E/neg.[b]	α ν	+	+	+	+	+	+	-	+	+	+
Secondary importance												
S. pyogenes	A	β (moderate)	±	-	+	+	+[c]	-[c]	-	-	+	-
S. zooepidemicus	C	β (wide)	±	-	+	+	+	-	-	-	-	+
S. species	G	β (wide)	±	-	+	+	+	-	-	-	±	-
S. species	L	β (wide)	-	±	±	+	±	-	-	-	+	-

[a] From Brown *et al.* (1969) "Microbiological Procedures for the Diagnosis of Bovine Mastitis." National Mastitis Council, Inc.

[b] Extracts of some strains of *S. uberis* react with Group E antisera but do not induce Group E antibodies in rabbits.

[c] Usual reaction.

Differentiation between the genera Staphylococcus and Micrococcus was accomplished by the anaerobic glucose fermentation test (O - F test). Isolants to be tested were grown for 24 hours at 37C on tryptone-yeast-extract agar. Medium for the test was autoclaved for 20 minutes at 115C and then steamed for 10 to 15 minutes to remove any dissolved oxygen. Solidification of the agar was accomplished by placing the tubes in iced water. A tube was immediately inoculated by placing a heavily inoculated wire loop down to the botton of the tube. The surface of the tube was covered with a 25 mm, or more, layer of a sterile parafin oil. Incubation was at 37C for 5 days. If the organism was a Staphylococcus, acid was produced anaerobically and the indicator changed to yellow throughout the tube. No color change or slight yellow color near the surface of the tube indicated a Micrococcus.

Tests for other microorganisms such as Coliforms, Pseudomonas, Corynebacteria, Diplococci, Bacilli, Mycobacteria, Nocardia, Mycoplasmas (PPLO), Fungi, and Prototheca were performed according to procedures set forth by the National Mastitis Council, Inc. (Brown et al., 1969). In this study, coagulase negative S. epidermidis, micrococci, and diphtheroids were considered nonpathogenic, and quarters harboring any of these microorganisms were considered not be infected.

Statistical Procedures

Chi square analyses (Snedecor and Cochran, 1971) of the number of recorded infections was performed to determine significant differences between treatments. Once a cow became infected, as determined by either observation of clinical mastitis or bacteriological tests,

neither that cow nor any of the quarters were included in subsequent time periods.

CHAPTER IV

RESULTS AND DISCUSSION

The results presented and the discussion concerning these results are on the data collected from the first 86 cows that have freshened in this trial. The study is continuing and further data to substantiate or detract from the conclusions made here are forthcoming.

No significant differences ($P > .05$) in the number of new infections occurring during the dry period and by 4 to 10 days after calving were found between treated and control cows that were not infected at drying off (Table V). Due to the relatively small number of cows making up each of the three lactation blocks, all lactations of negative cows at drying off were pooled to determine whether or not a difference existed between treated and control cows (Table VI). The number of cows (i.e., eight) with new infections determined by 4 to 10 days after calving were the same for each treatment. Therefore, no reduction in rate of new infection in negative cows was accomplished by the use of 500 mg benzathine cloxacillin per quarter over nontreated controls.

Since most normal lactating cows have four separate functional quarters it seems appropriate to discuss the effectiveness of dry cow therapy on a quarter basis as well as on a cow basis. Treated and control quarters (Table VII), that were negative at drying off, had a similar number of new infections by 4 to 10 days after freshening

TABLE V

NUMBER OF NEGATIVE COWS DEVELOPING INFECTIONS

Item	Lactation					
	One		Two		Three or more	
	Treated	Control	Treated	Control	Treated	Control
Number of negative dry cows	17	19[a]	15	14	9	9
Number of cows infected during the dry period	0	0	0	2	0	1
Number of additional cows infected at 4 to 10 days post-calving	4	2	2	3	2	0

[a]One cow with a blind quarter.

TABLE VI

COWS, NEGATIVE AT DRYING-OFF, POOLED

Item	All cows	
	Treated	Control
Number of negative dry cows	41	42[a]
Number of cows infected during the dry period	0	3
Number of additional cows infected at 4 to 10 days post-calving	8	5

[a]One cow with a blind quarter.

TABLE VII

NUMBER OF NEGATIVE QUARTERS DEVELOPING INFECTIONS

Item	Lactation					
	One		Two		Three or more	
	Treated	Control	Treated	Control	Treated	Control
Number of negative dry quarters	68	75[a]	60	56	36	36
Number of quarters infected during the dry period	0	0	0	2	0	1
Number of additional quarters infected at 4 to 10 days post-calving	7	5	2	7	3	0

[a]One blind quarter.

except for quarters of cows which had completed two lactations. Here, the number of infected quarters in treated and control (i.e., 2 versus 9) was significantly different ($P < .05$); however, the validity of any conclusions about these data would be questioned because of the small number of quarters involved. Lactation blocks were pooled to determine whether or not a difference existed between treated and control quarters of all negative quarters studied (Table VIII). There were more infections in the control quarters than in the treated quarters; however, this difference did not approach significance ($P > .50$).

One aspect of preventive mastitis research that deserves a great deal of attention when recommended procedures such as teat dipping during lactation, treatment of clinical infections, proper maintenance of equipment, and sound milking practices are followed is: "Does dry cow therapy reduce or prevent new infections in quarters that are negative at drying off?" In this trial, 12 of 164 (7.32%) treated quarters that were negative at drying-off contracted a new infection over the dry period. Pearson and Wright (1969) reported new infection rates of 14, 8.25, and 14%, respectively, for quarters treated with 300 mg procaine penicillin G, 250 mg of novobiocin and 300 mg of benzathine cloxacillin in three separate trials. A teat dip was used after the last milking of lactation in only one of these trials.

Kingwell et al. (1967) reported a new infection rate of 3.6% in quarters treated with either 1.0 g or 0.5 g of benzathine cloxacillin where teats were dipped with a 4% hypochlorite solution immediately after the last milking of lactation. Smith et al. (1967b) reported a 1.7% and 3.3% new infection rate where negative quarters were treated with 1.0 g and 0.2 g benzathine cloxacillin, respectively.

TABLE VIII

QUARTERS, NEGATIVE AT DRYING-OFF, POOLED

Item	All quarters	
	Treated	Control
Number of negative dry quarters	164	167[a]
Number of quarters infected during the dry period	0	3
Number of additional quarters infected at 4 to 10 days post-calving	12	12

[a]One blind quarter.

The rate of new quarter infection in nontreated controls in this trial was 9.12%. This agrees with results of other investigators who have reported new infection rates ranging from 15% (Oliver et al., 1956d) to 5.9% (Bratlie, 1972) (Table II). The variation in new infection rate may be due in part to the system of sampling after freshening or to the criteria established for an infection in each of the trials.

By comparing the percentage of treated quarters (7.32%) to the percentage of control quarters (9.12%) that became infected, there is a 19.7% reduction (not statistically significant) in new infection rate by treating quarters that are negative at drying-off. However, as noted earlier there was no difference at all between the treated and control groups, when considered on the basis of number of cows infected. Pearson and Wright (1969) reported a non-significant 12.5% reduction, in new infection rate by treating negative quarters with 300 mg procaine penicillin G during the dry phase.

New Infections in Early Lactation

Efforts were made to determine the effect of dry cow treatment with 500 mg of benzathine cloxacillin on the occurrence of new infections during the first month of the ensuing lactation. Two of 28 treated cows developed additional infections during the first month of lactation as did two of 29 control cows. It appears that in this trial there was no additional preventive effect, during early lactation, by the use of dry cow therapy.

Teat Dipping

After approximately four months of this trial, teat dipping cows

once a day for seven days after drying off was begun as a management procedure (Table IX). Of the 41 negative cows treated in this trial, 22 were teat dipped as described above and none of these 22 developed new infections over the dry period or by 4 to 10 days post-calving. Nineteen of the 41 treated cows were not teat dipped. Eight of these, involving 12 quarters, developed new infections by 4 to 10 days post-calving (Table X).

Similar results were found for the 42 non-treated control cows. Twenty-one of the control cows were teat dipped and one (involving one quarter) developed an infection by 4 to 10 days post-calving. An equal number of control cows (21) were not teat dipped and of these seven, involving 14 quarters, developed new infections (Table X). A highly significant difference ($P < .01$) was found between teat dipped and not teat dipped cows and quarters.

Oliver, Dodd and Neave (1956c) reported similar results in an experiment where teats of cows not infused with antibiotics were dipped after the last milking of lactation and 24 hours later in a 5% iodine solution. They reported that 8 of 25 teat dipped cows developed new infection during the early dry period and 18 of 25 non-teat dipped cows developed infections. Fifteen of 100 teat dipped quarters became infected and 28 of 100 quarters not teat dipped developed infections in the dry period. Differences for both cows and quarters were highly significant ($P < .01$).

A factor that may have influenced the results attributed to teat dipping in the present experiment was that a change in milking facilities from stanchion to a "double three-in-line" parlor, was initiated at approximately the same time as the teat dipping regime. The milking

TABLE IX

EFFECT OF TEAT DIPPING[a] FOR ONE WEEK AFTER DRYING OFF ON NUMBER OF COWS BECOMING INFECTED

Item	Treated (41)[b]		Control (42)[b]	
	Teat dipped	Not teat dipped[c]	Teat dipped	Not Teat dipped[c]
Number of negative cows	22	19	21	21[d]
Number of cows infected during the dry period	0	0	0	3
Number of additional cows infected at 4 to 10 days post-calving	0	8	1	4
Total number of cows infected[e]	0	8	1	7

[a]"Bovadine," West Agro-Chemical Products, Inc., Subsidary of West Chemical Products, Inc., Long Island, New York.

[b]Number of cows. No significant difference existed between treated and control groups.

[c]The first 40 cows assigned to the trial were not teat dipped.

[d]One cow with a blind quarter.

[e]Significant differences ($P < .01$) were found between total teat dipped and total non-teat dipped cows.

TABLE X

EFFECT OF TEAT DIPPING[a] FOR ONE WEEK AFTER DRYING-OFF ON NUMBER OF QUARTERS BECOMING INFECTED

Item	Treated (164)[b]		Control (167)[b]	
	Teat dipped	Not teat dipped[c]	Teat dipped	Not teat dipped[c]
Number of negative dry quarters	88	76	84	83[d]
Number of quarters infected during the dry period	0	0	0	3
Number of additional quarters infected at 4 to 10 days post-calving	0	12	1	11
Total number of quarters infected[e]	0	12	1	14

[a]"Bovadine," West Agro-Chemical Products, Inc., Subsidary of West Chemical Products, Inc., Long Island, New York.

[b]Number of quarters. No significant difference existed between treated and control groups.

[c]The first 40 cows assigned to the trial were not teat dipped.

[d]One blind quarter.

[e]Significant differences ($P < .01$) were found between total teat dipped and total non-teat dipped quarters.

facility change may be confounded in the teat dipping results, in that better milking procedure was possible in the parlor. However, of the 23 quarters not teat dipped which developed an infection by 4 to 10 days post-calving (Table X), 22 were determined to be infected by the presence of clinical mastitis within five days of calving. This suggests that the new infections were probably present at calving, and not caused by milking procedures.

The major protion of new infections were determined by observation of clinical mastitis in this trial (Table XI). Eighty-five percent of the mastitis infections in cows were determined by clinical observations and 90.3% of infected quarters were detected by this means.

Types of Bacteria Isolated

Treating cows with 500 mg benzathine cloxacillin at drying-off does not appear to influence the type or proliferation of certain bacteria in preference to others, on the basis of strains identified from infected cows (Table XII).

Cows Infected with *Staphylococcus aureus* at "Drying-Off"

In this trial, provision was made to evaluate dry cow therapy in regard to its ability to eliminate infections that were present at drying-off. Preliminary studies showed that the number of quarters infected with an organism other than *S. aureus* would be too small for statistical analysis. At this time, only three cows that had one or more quarters infected with *S. aureus* at drying-off have calved and can be discussed. All three of these cows were randomly assigned to

TABLE XI

INFECTIONS DETERMINED BY BACTERIOLOGICAL OR CLINICAL MEANS

Method	No.	%	Total clinical	Total bacteriological
Cows				
Bacteriological only	3	15.0	-----	55.0%
Bacteriological and clinical	8	40.0	85.0%	-----
Clinical only	9	45.0		
Quarters				
Bacteriological only	3	9.7	-----	38.7%
Bacteriological and clinical	9	29.0	90.3%	-----
Clinical only	19	61.3		

TABLE XII

TREATMENT EFFECT ON TYPE OF BACTERIA ISOLATED

Organism	Treated Quarters[a]	Control Quarters[a]
Staphylococcus aureus	1	0
Streptococcus agalactiae	0	0
Streptococcus dysgalactiae	0	2
Streptococcus uberis	1	2
Streptococci species	1	1
Pseudomonas	1	0

[a]Number of quarters from which bacteria was isolated during the dry period or by 4 to 10 days post-calving.

the control group. At 4 to 10 days post-calving two of the cows remained infected in the same quarters as at drying-off. The five infected quarters had three S. aureus infections and two Streptococcus agalactiae infected quarters, one of which was previously infected at drying-off with Streptococcus agalactiae. The remaining cows had one quarter infected at drying-off. This animal was negative at 4 to 10 days post-calving, but developed clinical mastitis in an adjacent quarter shortly after the 4 to 10 days post-calving samples were complete.

CHAPTER V

SUMMARY AND CONCLUSIONS

This experiment was undertaken to evaluate the effects of drug therapy during the dry period on mastitis in dairy cattle under the climatic and management conditions prevailing in the southwestern United States.

The cows were assigned to treatments according to a randomized block design, blocking on status of infection and lactation number near the end of lactation. Treatments were 500 mg of benzathine cloxacillin per quarter infused after the last milking at the end of lactation and a non-treated control. Infections were determined by bacteriological tests and the presence of clinical mastitis. Incidence of infection was determined one week prior to drying-off, 4 to 10 days after calving, one month after calving and whenever clinical mastitis was observed during this time span.

No significant difference ($P > .05$) in new infection rate was found between treated and control cows or quarters that had no infections at drying-off. Incidence of new infection was 7.32% in treated quarters and 9.12% in control quarters. Teat dipping for seven days after drying-off caused a significant reduction ($P > .01$) on the number of cows and quarters becoming infected through 4 to 10 days post-calving. Clinical manifestations accounted for 85% of all infections detected in cows and 90.3% of all infected quarters detected.

It appears that dry cow therapy, using 500 mg benzathine cloxacillin in quarters that are free of infection at drying-off, has little benefit in reducing the incidence of new infection during the nonlactating period. Dipping of teats for seven days after drying-off using a recommended disinfectant apparently reduced new dry period infections.

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